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## SOME OBSERVATIONS ON PHAGOCYTOSIS OF DIPH- THERIA BACILLI\*

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CONTRARY to the statement of Wright and Douglas<sup>1</sup> that *B. diphtheriae* is not readily taken up by the leucocytes in normal serum, the strains under my observation were found very susceptible to phagocytosis. When a rather thick suspension of bacilli is incubated for about one hour with an equal quantity of normal defibrinated human or dog blood, as many as 50, or even more, bacilli may be taken up by a single leucocyte. Two strains of *B. diphtheriae*, both virulent to guinea-pigs, were tested. Leishman's quantitative method of estimating the degree of phagocytosis was employed. As a rule, the various mixtures were kept in the incubator for one hour. When more than about 10 bacilli are taken up by a single leucocyte it is difficult to make an accurate count, especially when the bacilli have become fragmented or only the granules are distinctly stained. Hence rather thin suspensions were usually found preferable. From 20 to 50 leucocytes were counted to get the average number of bacilli taken up.

1. When the blood serum is removed by washing in normal salt solution, phagocytosis is greatly diminished or almost absent, depending chiefly on the thoroughness of the washing. Four or even five washings are required. Even after such thorough washings a few leucocytes often appear to have taken up a considerable number of bacilli, while the majority are quite inactive. This is oftenest found when very concentrated suspensions are used. It often appears as if a *clump* of bacilli—the presence of a few clumps is difficult to avoid in preparing suspensions of *B. diphtheriae*—had settled upon the leucocyte, rather than that such a number had actually been taken up by the leucocyte.

In the following set of experiments, in each of which a different blood and a different suspension of bacilli were used, a portion of

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<sup>1</sup> *Proc. Roy. Soc.*, London, 1903, 72, p. 357.

the blood was washed four or five times, and enough normal salt solution was added to make up the same bulk as before washing; 0.3 c.c. of normal blood and washed blood were then placed in separate tubes and incubated with equal amounts of the same bacterial suspension.

HUMAN BLOOD.				DOG BLOOD.			
Unwashed			Washed	Unwashed			Washed
33.00	.	.	5.70	12.45	.	.	4.00
17.30	.	.	5.40	6.50	.	.	0.02
5.25	.	.	0.76	3.10	.	.	0.00
11.30	.	.	0.80				

2. When normal serum is added to washed leucocytes, phagocytic activity is largely restored. This does not occur if the serum has been previously heated to 58 to 60° C. for 15 to 30 minutes.

	PHAGOCYTOSIS			
	Unwashed	Washed	Washed plus Normal Serum	Washed plus Heated Serum
Dog blood.....	6.00	0.20	2.45	0.60
Human blood.....	11.30	0.80	8.50	0.10

3. When the bacilli have been suspended in normal serum for 30 minutes at 37° C., the serum being subsequently removed by washing they undergo phagocytosis by washed leucocytes. The bacilli are not thus sensitized by serum which has been heated at 58° to 60° C. for 30 minutes. It was found that when the bacilli were treated with normal serum, there was an average phagocytosis of 9.5 by washed dog leucocytes, while the same quantity of the same bacterial suspension, when treated by the same quantity of heated serum gave a phagocytosis of only 0.6. Similarly with washed human leucocytes, two different suspensions treated with normal serum gave respectively a phagocytosis of 7.0 and 23.4, while, under identical conditions, treatment with heated serum gave 0.1 and 4.5 per leucocyte.

4. E. L. Walker<sup>1</sup> reported that when diphtheria bacilli are heated at 70° to 100° C., there is a decrease in phagocytosis, and he ascribed this to a destruction of the toxin by heat. An attempt was made to repeat the experiment, and, if the result had been verified, it was

<sup>1</sup> "The Relative Influence of the Blood Fluids and the Bacterial Toxins on Phagocytosis," *Jour. Med. Res.*, 1905, 14, p. 173.

planned to restore phagocytosis by means of diphtheria anti-toxin. However, no marked decrease in phagocytosis was observed after heating the bacilli. Heating the bacilli at 70° to 75° C. for one-half hour, and then incubating with normal human blood, gave the following results, smears being examined at the end of every hour for four hours:

	1 Hr.	2 Hrs.	3 Hrs.	4 Hrs.
Unheated bacilli.....	19.6	31.0	50.0?	75.0?
Heated ".....	13.5	22.0	20.0	35.0

It will be seen that after one hour's incubation there is only a slight difference in the phagocytosis of the heated and unheated bacilli. The greater differences after longer periods of incubation are probably due to an increase in the number of the unheated bacilli, and a consequent real or apparent greater phagocytosis. Heated bacilli also often stain poorly, and this may result in a lower count; possibly some are entirely destroyed by prolonged heating.

When rather concentrated suspensions were used, so many bacilli were taken up, even after heating to the boiling-point, that an accurate count was found impossible, as in the following experiment: Similar quantities of a uniform suspension were heated 15 minutes each, at temperatures varying from 60° to 100° C., and then incubated one hour with normal blood; there was no apparent increase or decrease in phagocytosis as a result of the heating; even the bacilli that had been boiled were taken up in such numbers that an accurate count was impossible. This experiment was repeated several times with *B. diphtheriae* and also with *St. aureus*.

5. Some tests were made of the opsonic power of the blood serum of diphtheria patients. Two series of tubes were prepared, each tube containing 0.2 c.c. of a suspension of washed human blood and 0.2 c.c. of a suspension of diphtheria bacilli; then to one series were added falling quantities of normal human serum, to the other series similar quantities of serum from the diphtheria patient, enough normal salt solution being added to make up a total of 0.6 c.c. in each tube. Serum from a patient seven days ill, with temperature slightly above the normal and a few bacilli still in the throat, gave the following result:

	Normal Serum	Serum from Diph- theria Patient
0.2 c.c. serum.....	44.0	34.0
0.1       ".....	37.0	29.0
0.05       ".....	37.5	28.3
0.025       ".....	28.5	19.5
0.006       ".....	22.0	24.2

There appears to have been some decrease in opsonin at this time. After another week there was a further decrease; the patient exhibited some symptoms of pulmonary tuberculosis (a positive diagnosis being made soon after), and no further tests were made in this case.

The serum of another patient was tested after two weeks' illness. The temperature was normal; bacilli were still found on the tonsils. In this case a rather marked increase in opsonin was present.

	Normal Serum	Serum from Diph- theria Patient
0.2 c.c. serum.....	4.20	6.05
0.1       ".....	3.60	4.02
0.05       ".....	2.30	3.30
0.025       ".....	2.90	4.10
0.006       ".....	1.00	2.20

It would be of interest to determine conclusively whether or not there is an increase in opsonin during convalescence from such a disease as diphtheria, where the infective organisms multiply only locally, while the toxins invade the general system. This, if demonstrated, would tend to show that some substance is split off by the bacteria which enters the circulation and stimulates the formation of opsonin. It would be desirable to follow several cases with this end in view through the whole course of the disease.

It might also be of interest to follow the opsonic index of the horse or other animal during a process of immunization with diphtheria toxin. Two such observations have been made on the horse, one during the earlier course of the immunization, the other after some degree of immunization had been established. The first observation, conducted in the same way as the experiments on the human patients, showed a decrease in opsonin, the index being approximately 0.5. In the second the index was found to be about 1.0.

## CONCLUSIONS.

1. *B. diphtheriae* is very susceptible to phagocytosis.
2. Phagocytosis is not materially affected by heating the bacilli, as claimed by Walker, and it has not been shown that the diphtheria toxin favors phagocytosis.
3. There is probably an increased formation of opsonin during convalescence from diphtheria, but this point requires further investigation.